جامعة نيويورك أبوظبي

NYU | ABU DHABI

Targeted delivery of anticancer therapeutics

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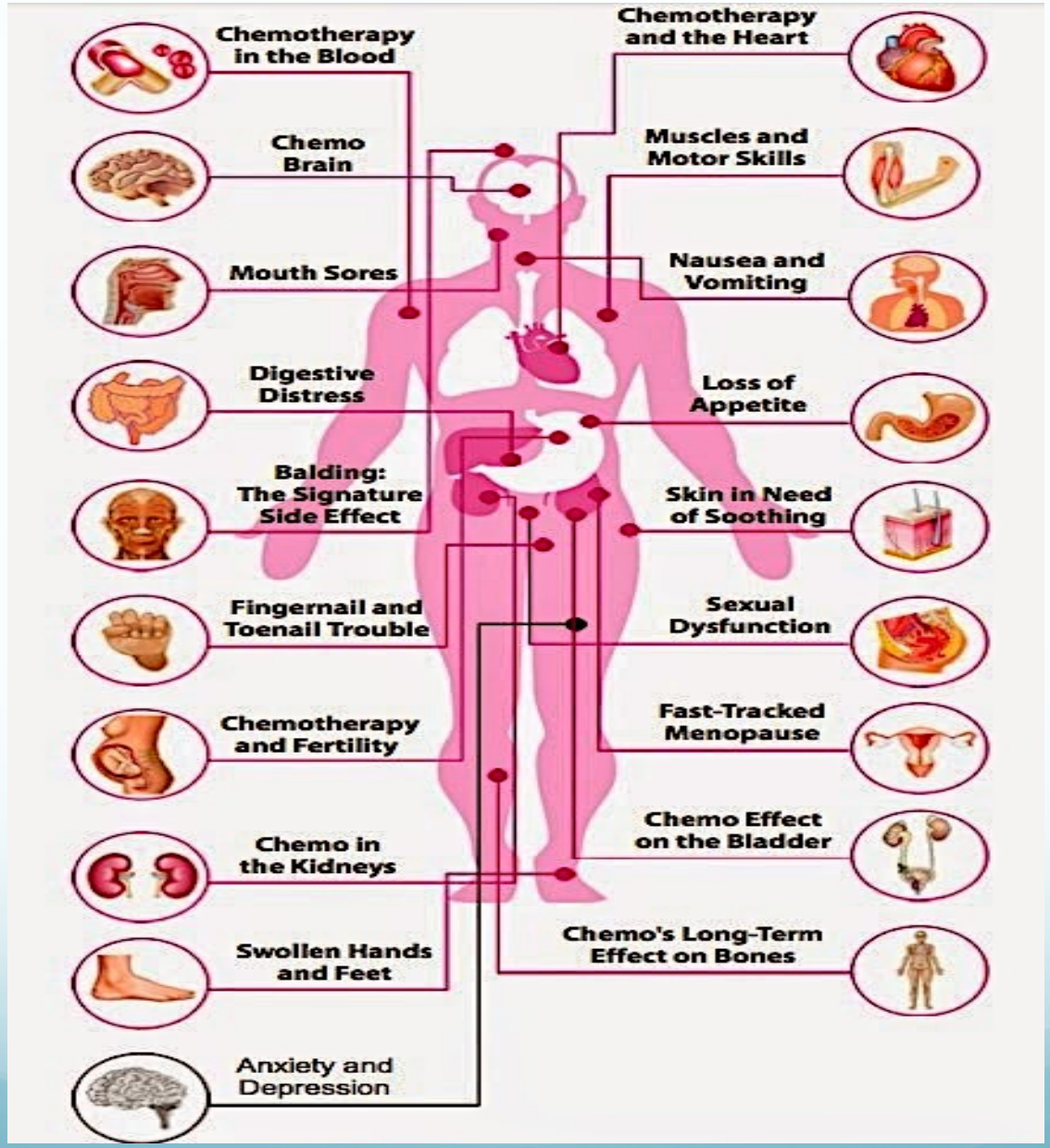
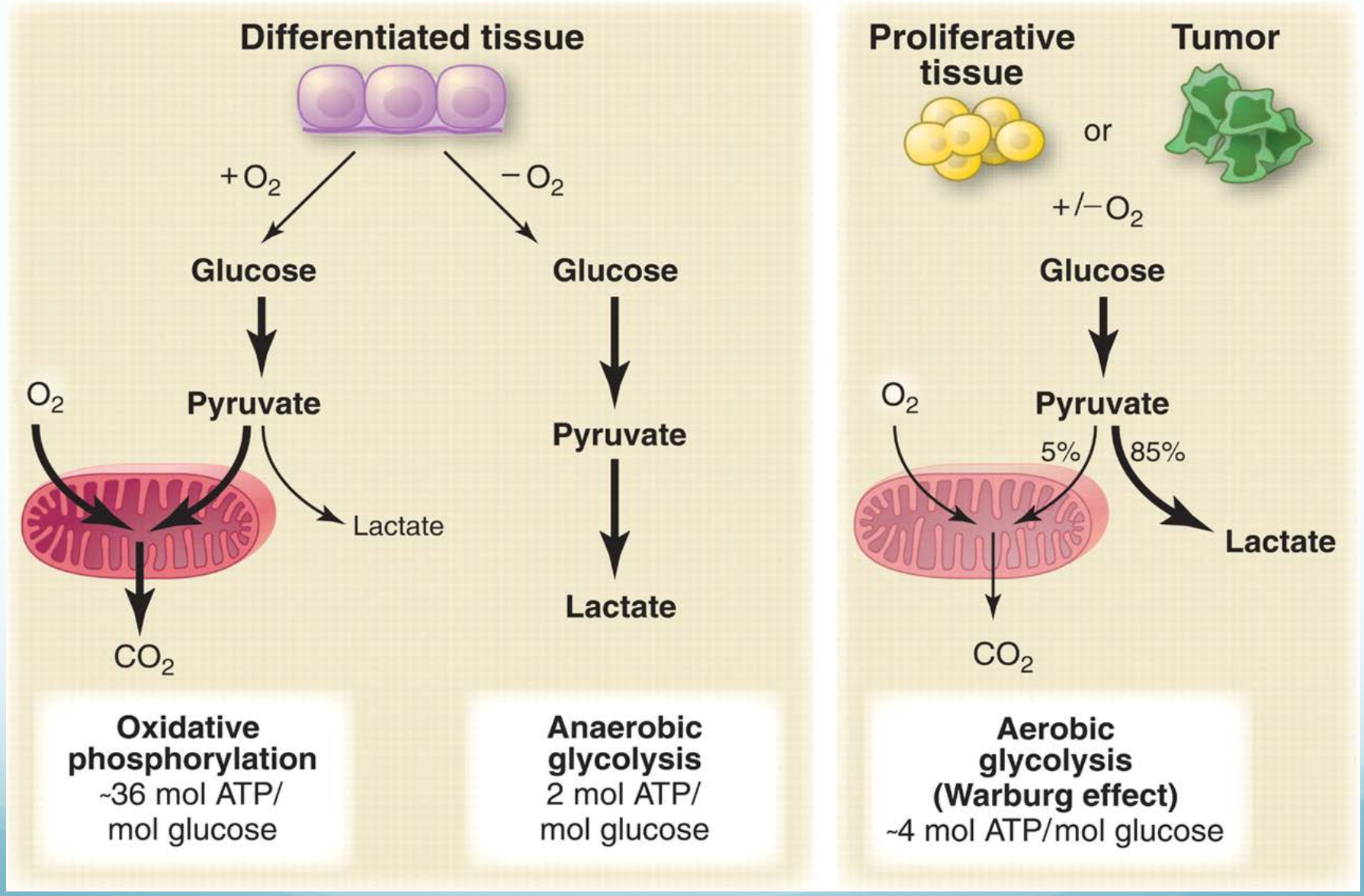
Side-effects of chemotherapy



Metabolism of healthy vs cancerous cells



(Vander Heiden et al., 2009)

Hexokinase 2 (HKII)

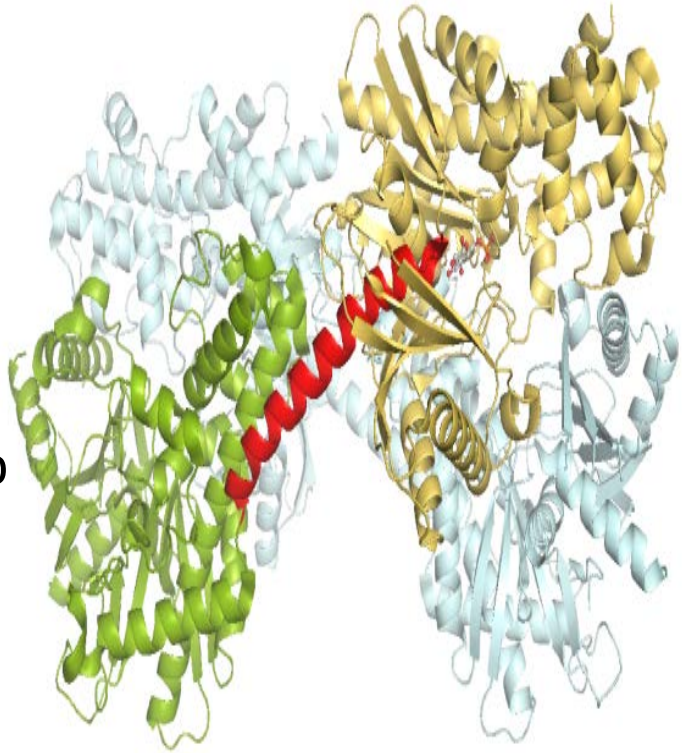
- Catalyzes the first step of glycolysis:
  glucose ➔ glucose 6-phosphate

- The predominant isoform over-expressed in malignant tumors

  in highly aggressive cancer cells HKII levels > 100-fold higher than normal cells

- In tumors, up to 70% of the enzyme is bound to the outer mitochondrial membrane (OMM) via interaction with the voltage dependent anion channel (VDAC).

- Interaction with VDAC occurs via the **N-terminal 15-amino acids** of HKII.

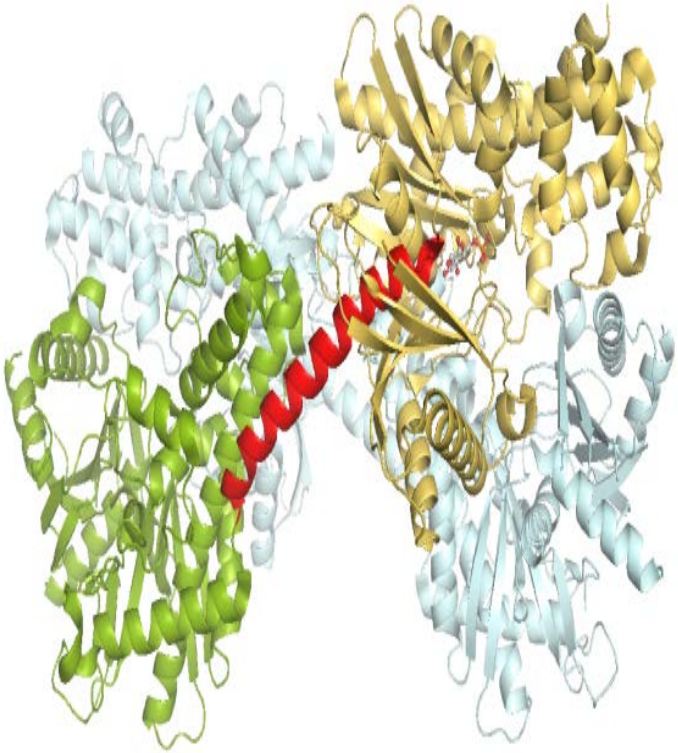


(Rabeh et al., 2006)

Hexokinase 2 (HKII)

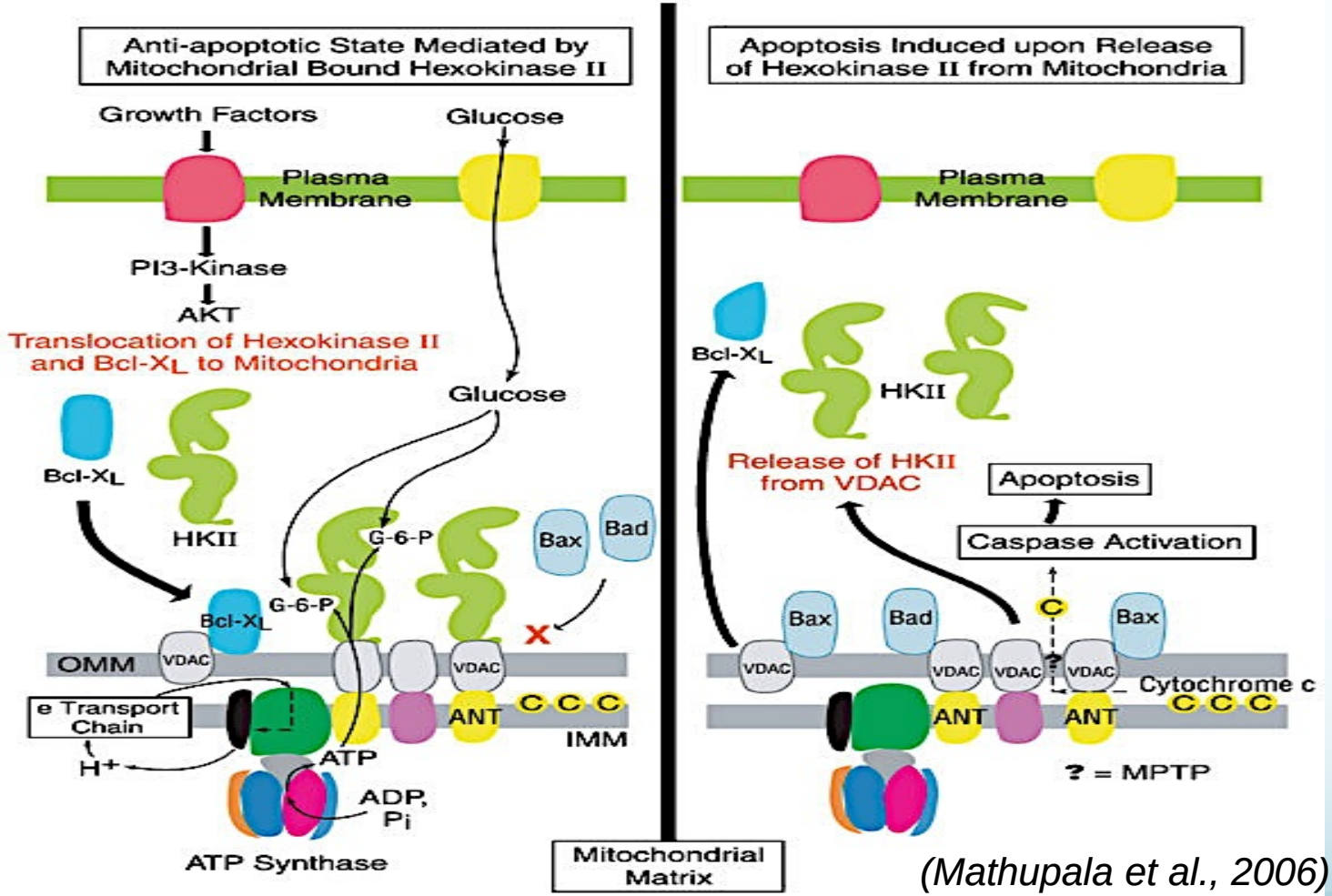
- Mitochondrial binding gives HKII preferential access to mitochondria-generated ATP, which the enzyme selectively uses for glucose phosphorylation.

- The glucose-6-phosphate product of HKII-mediated phosphorylation of glucose is a metabolic intermediate precursor in most biosynthetic pathways
    - **essential for generating the proteins, nucleic acids and lipids required for cell proliferation**.



(Rabeh et al., 2006)

Mitochondria-bound HKII plays a major role in preventing tumor apoptosis



(Mathupala et al., 2006)

HKII is required for tumor initiation and maintenance, as well as promotion of metastasis.

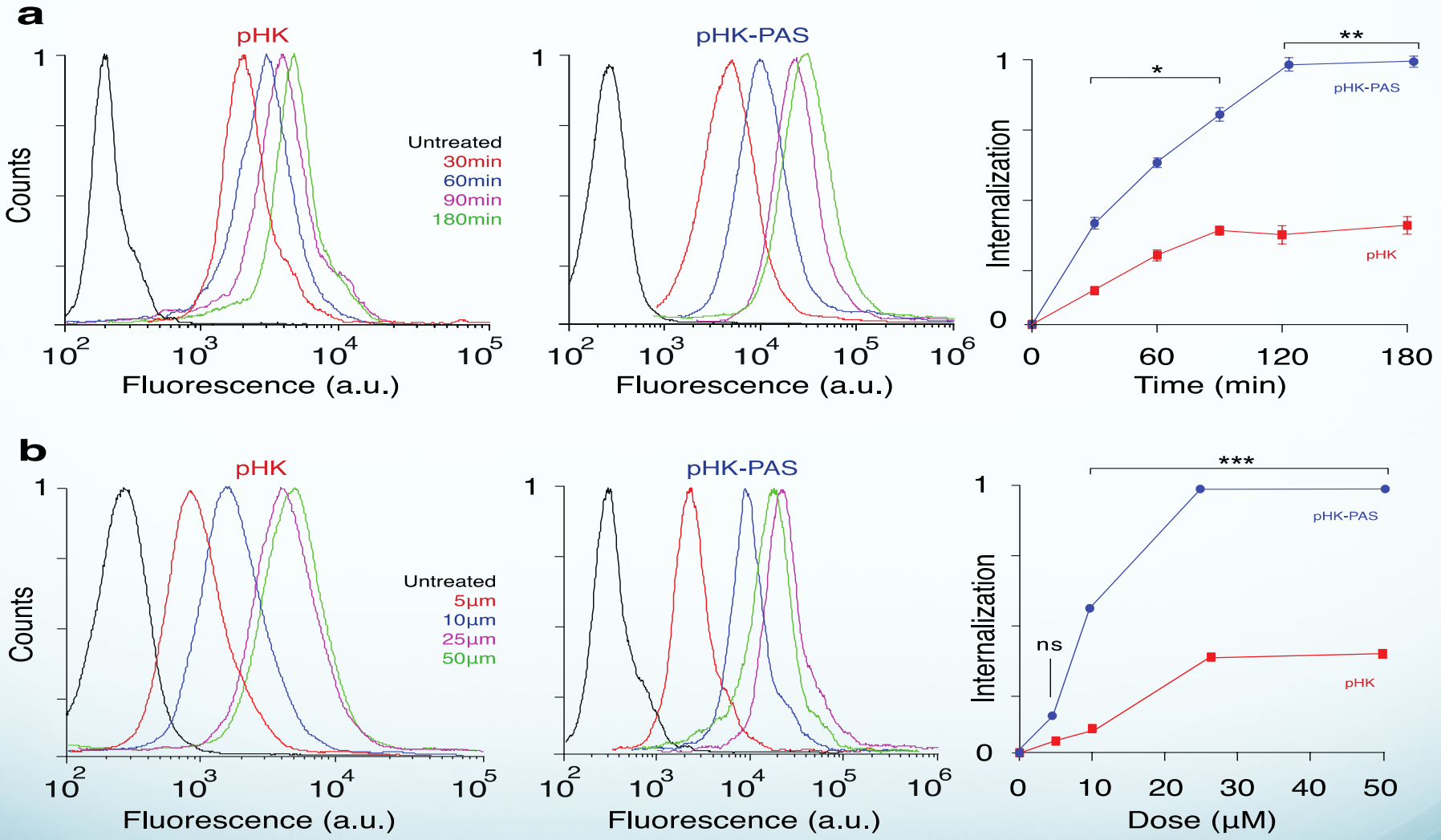
Hypothesis

A peptide corresponding to the VDAC-binding N-terminal 15 amino acids of HKII (pHK) can be used to selectively dissociate HKII from mitochondria, and subsequently inhibit glycolysis and induce apoptosis, in cancer cells.

pHK	MIASHLLAYFFTELN-amide
pHK_{A488}	A488-MIASHLLAYFFTELN-amide
pHK-PAS	MIASHLLAYFFTELNFFLIPKG-amide
pHK-PAS_{A488}	A488-MIASHLLAYFFTELNFFLIPKG-amide

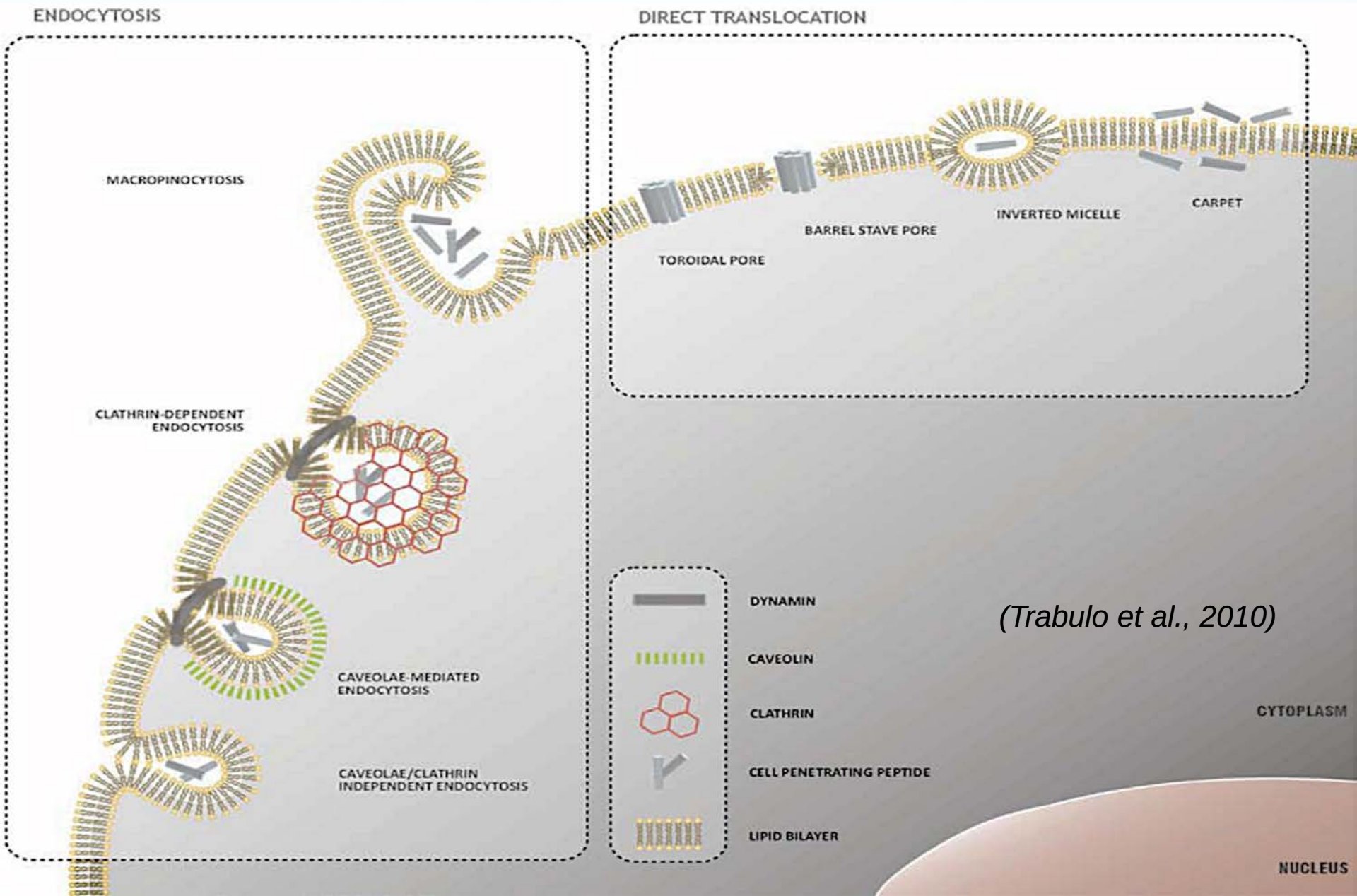
PAS: Penetration Accelerating Sequence

Quantification of cellular uptake



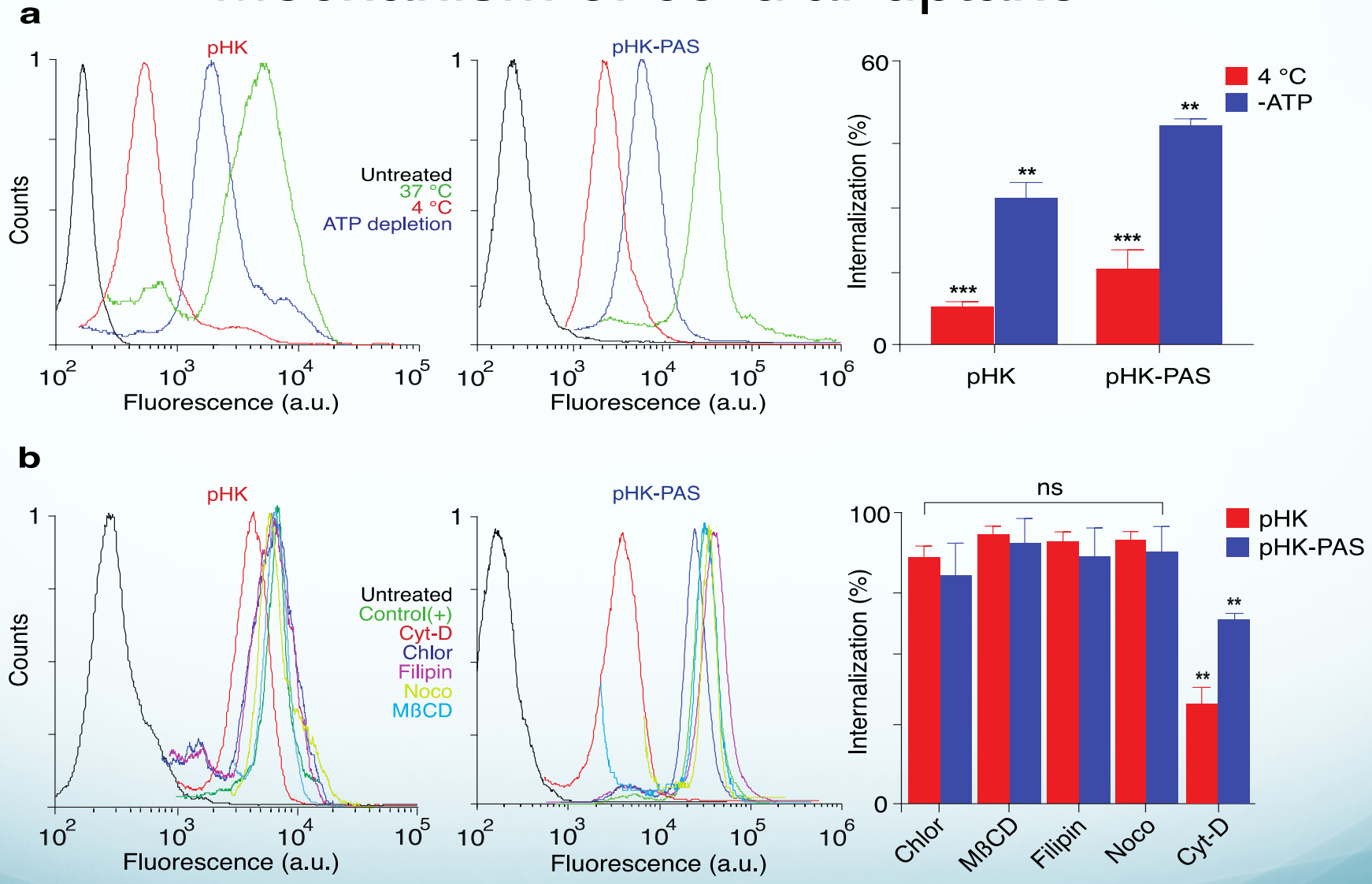
pHK-PAS functions as a cell-penetrating peptide (CPP).

Proposed mechanisms of CPP internalization



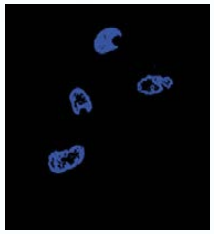
(Trabulo et al., 2010)

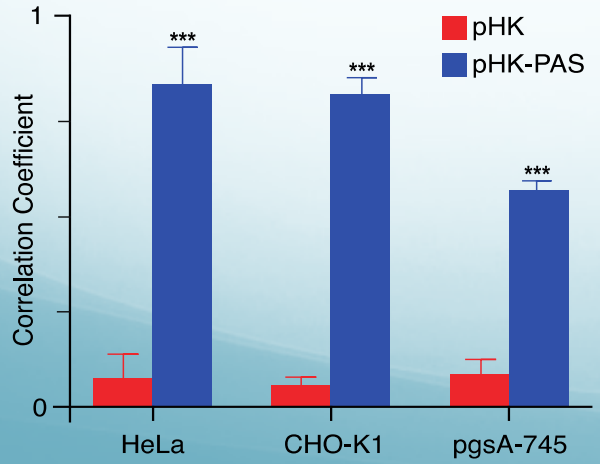
Mechanism of cellular uptake



pHK-PAS enters cancer cells by both endocytosis (macropinocytosis) and an energy-independent mechanism.

Intracellular localization





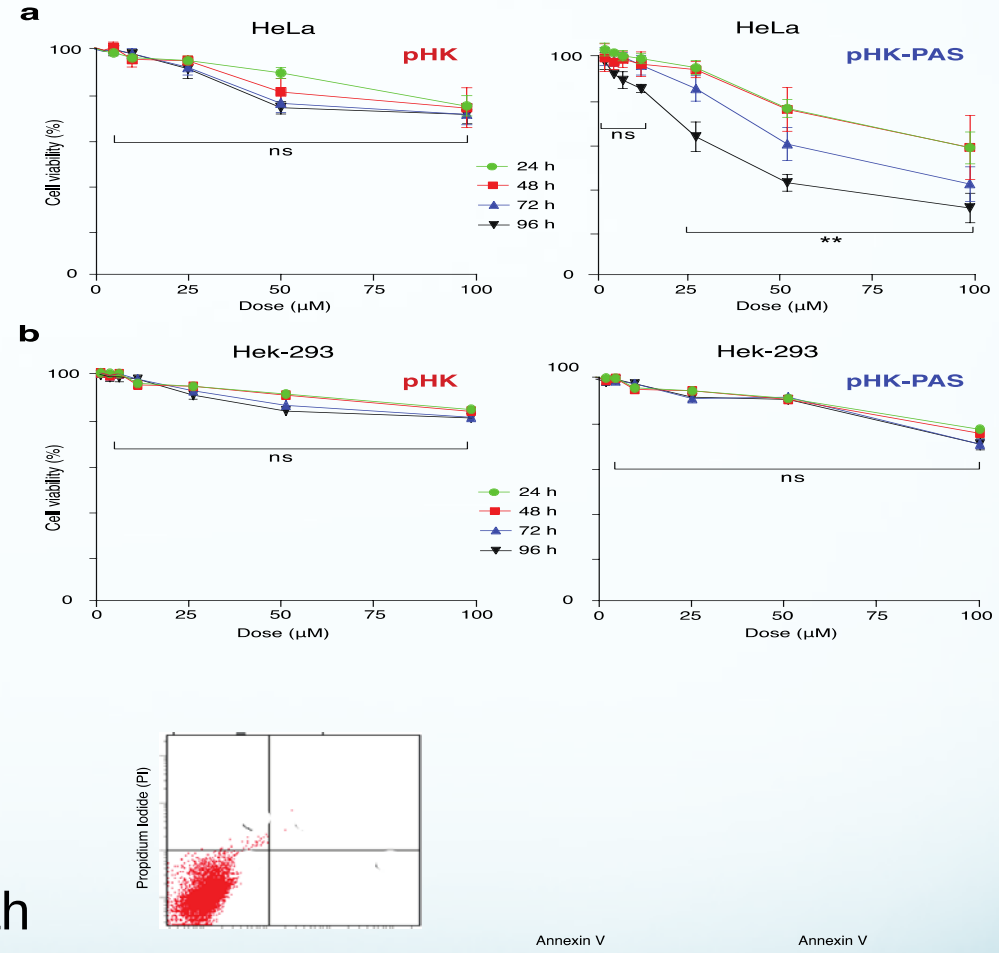
pHK-PAS CPP exhibits substantially greater mitochondrial localization compared to pHK.

Toxicity of the HKII-derived peptides

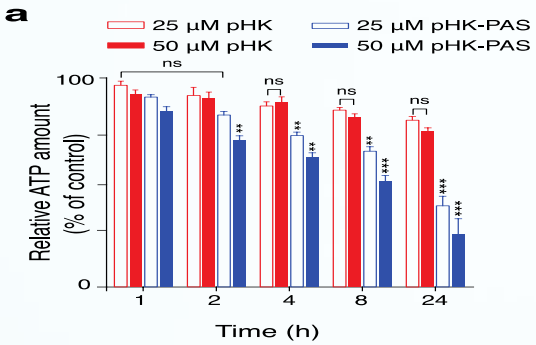
pHK-PAS is significantly more toxic to cancer cells compared to pHK.

pHK-PAS induces substantially lower toxicity in non-cancer HEK-293 cells vs cancer cells.

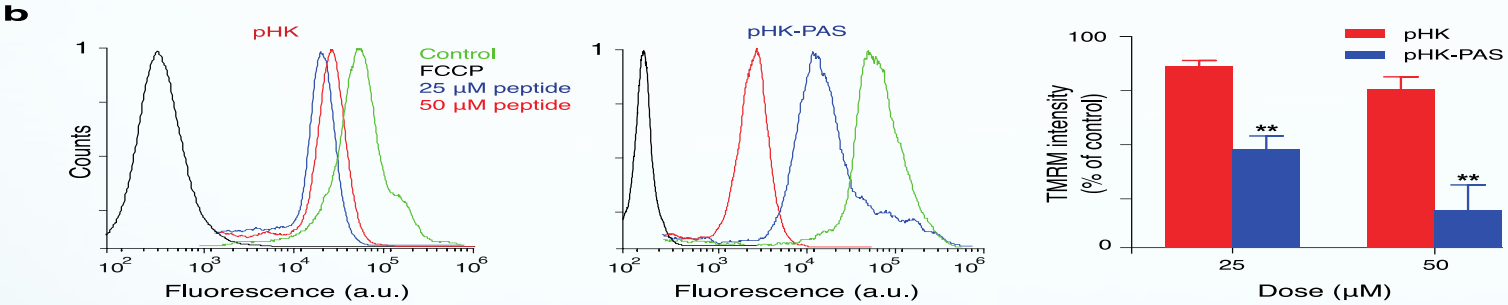
pHK-PAS-induced cancer cell death occurs primarily via apoptosis.

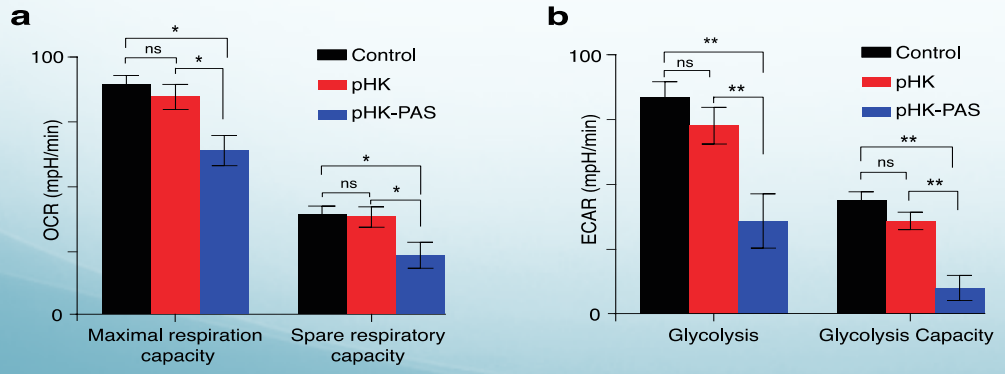


Mitochondrial membrane potential ($\Delta\Psi_m$), intracellular ATP levels and cellular metabolic activities



Treatment of cancer cells with pHK-PAS depolarizes $\Delta\Psi_m$ and depletes intracellular ATP levels.



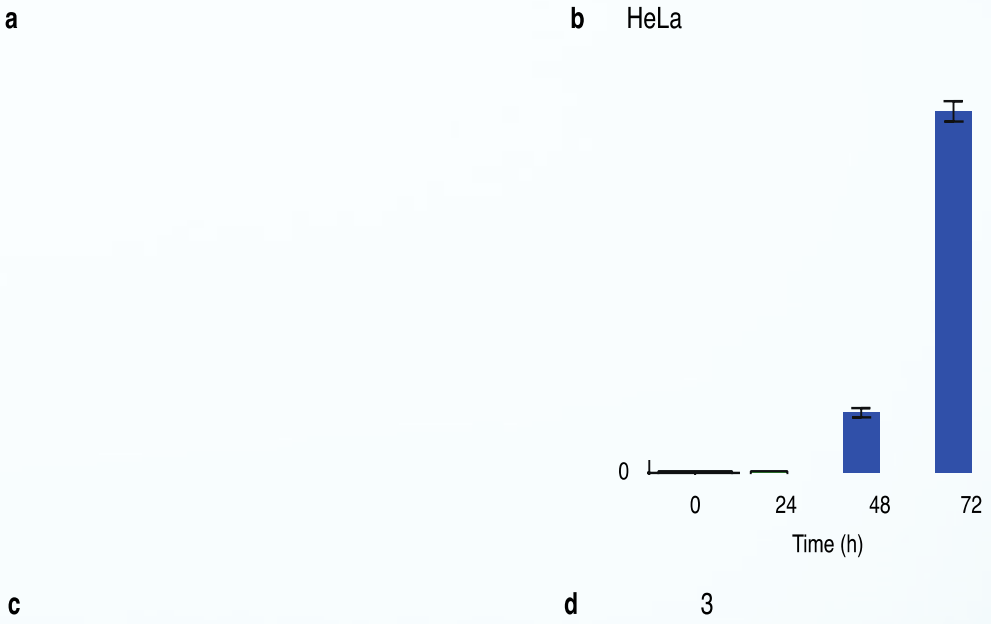


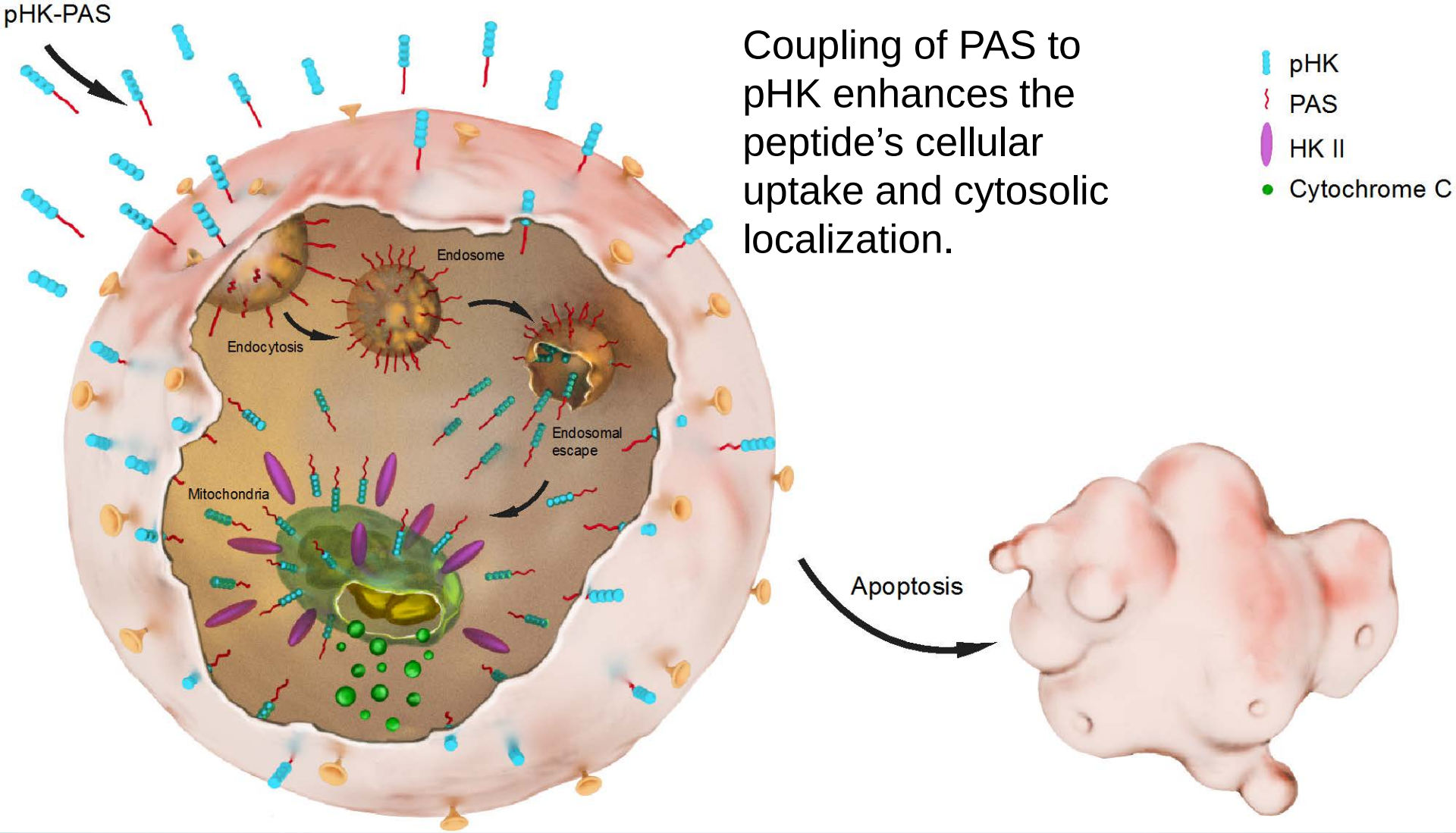
- pHK-PAS leads to impairment of mitochondrial respiration.
- pHK-PAS also strongly inhibits glycolytic function.

HKII content of mitochondrial and cytosolic fractions of HeLa cells

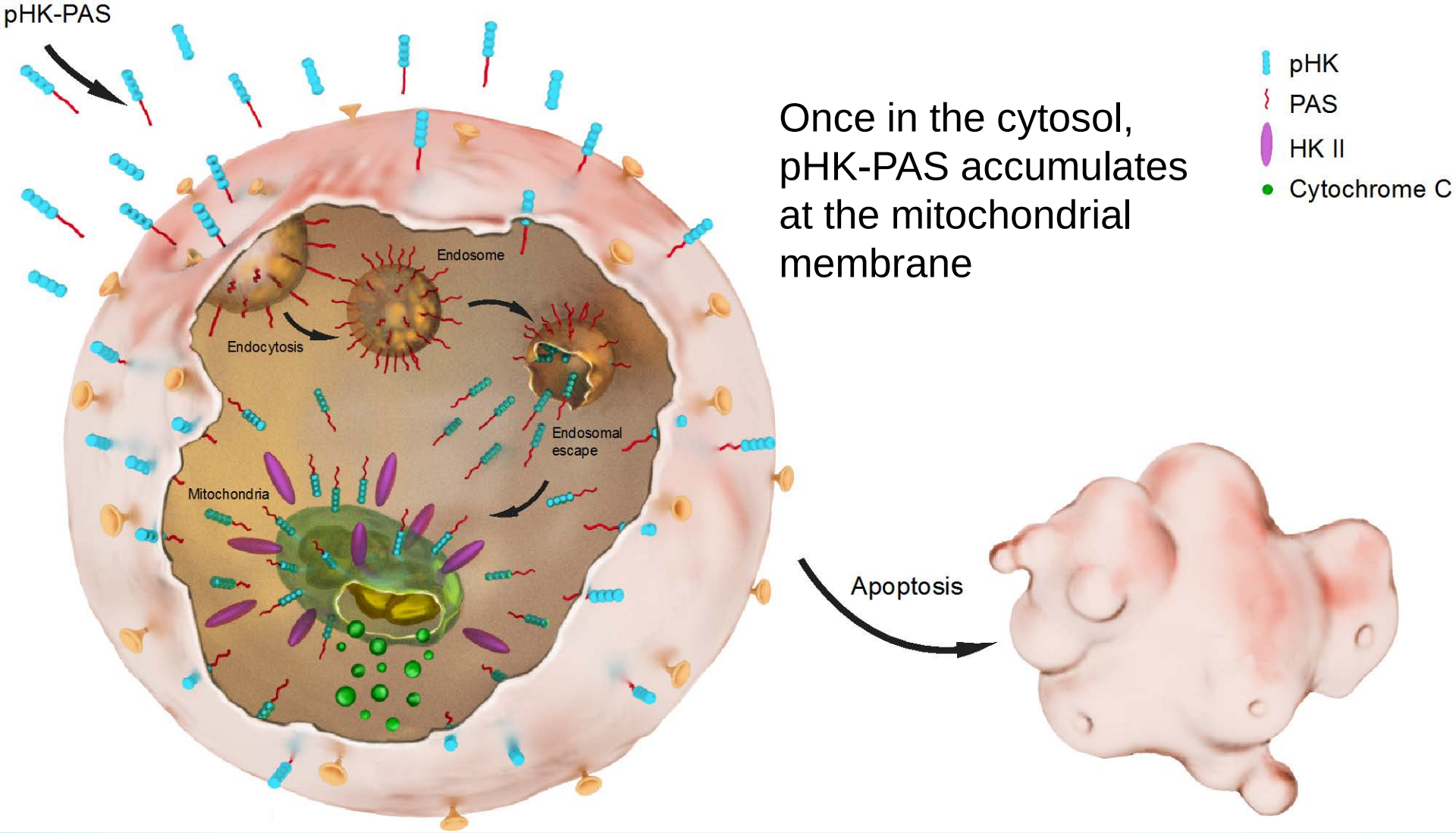
In HeLa cells, pHK-PAS displaces HKII from mitochondria, which triggers release of cytochrome c to the cytosol and apoptosis.

In HEK-293 cells, displacement of the lower levels of endogenous HKII from mitochondria does not trigger apoptosis.

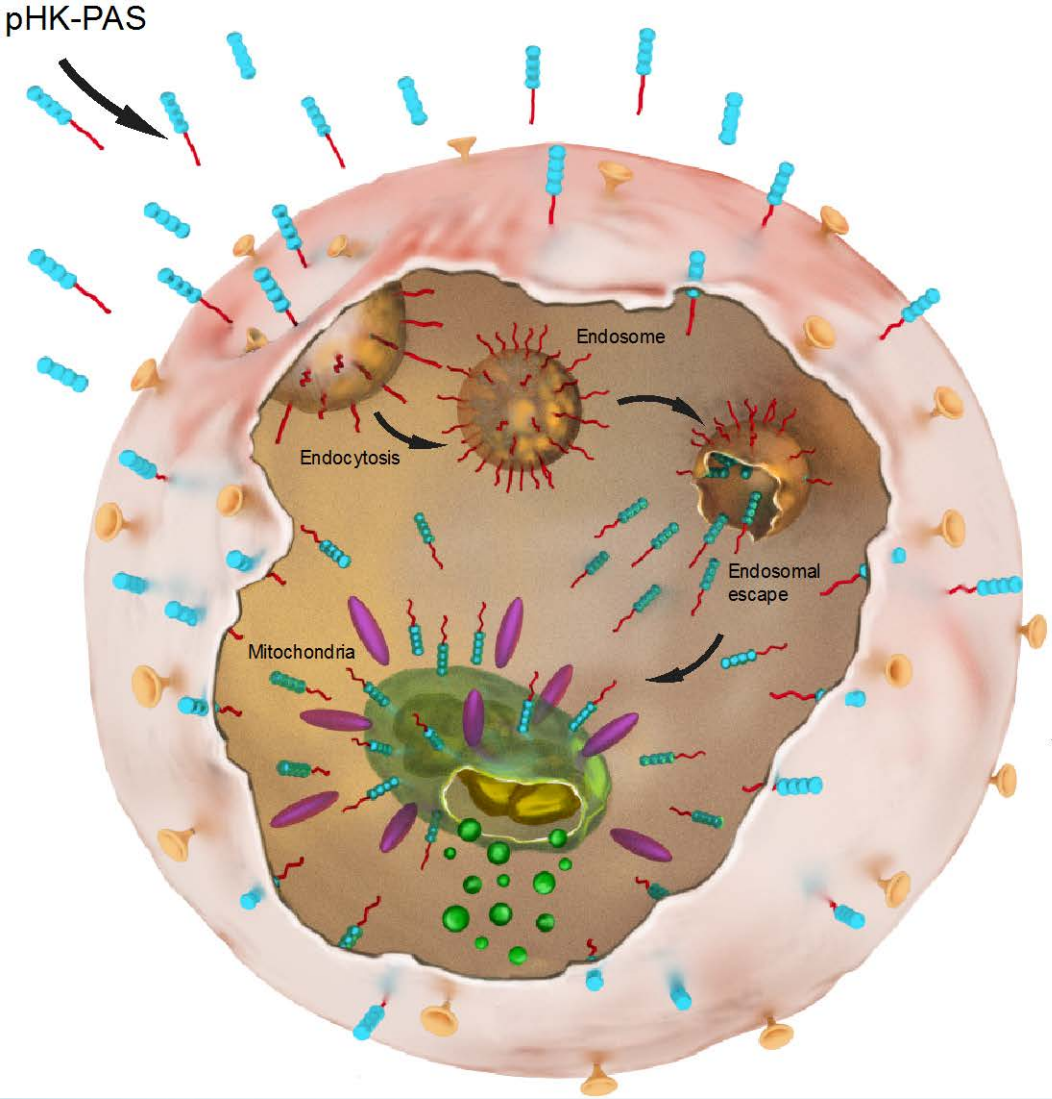




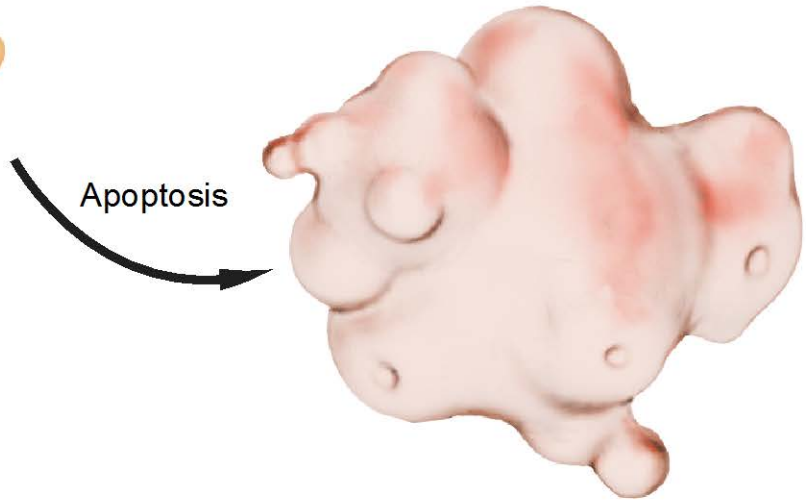
Coupling of PAS to pHK enhances the peptide's cellular uptake and cytosolic localization.

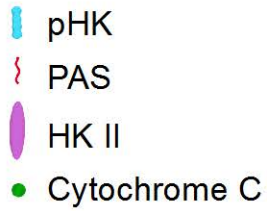


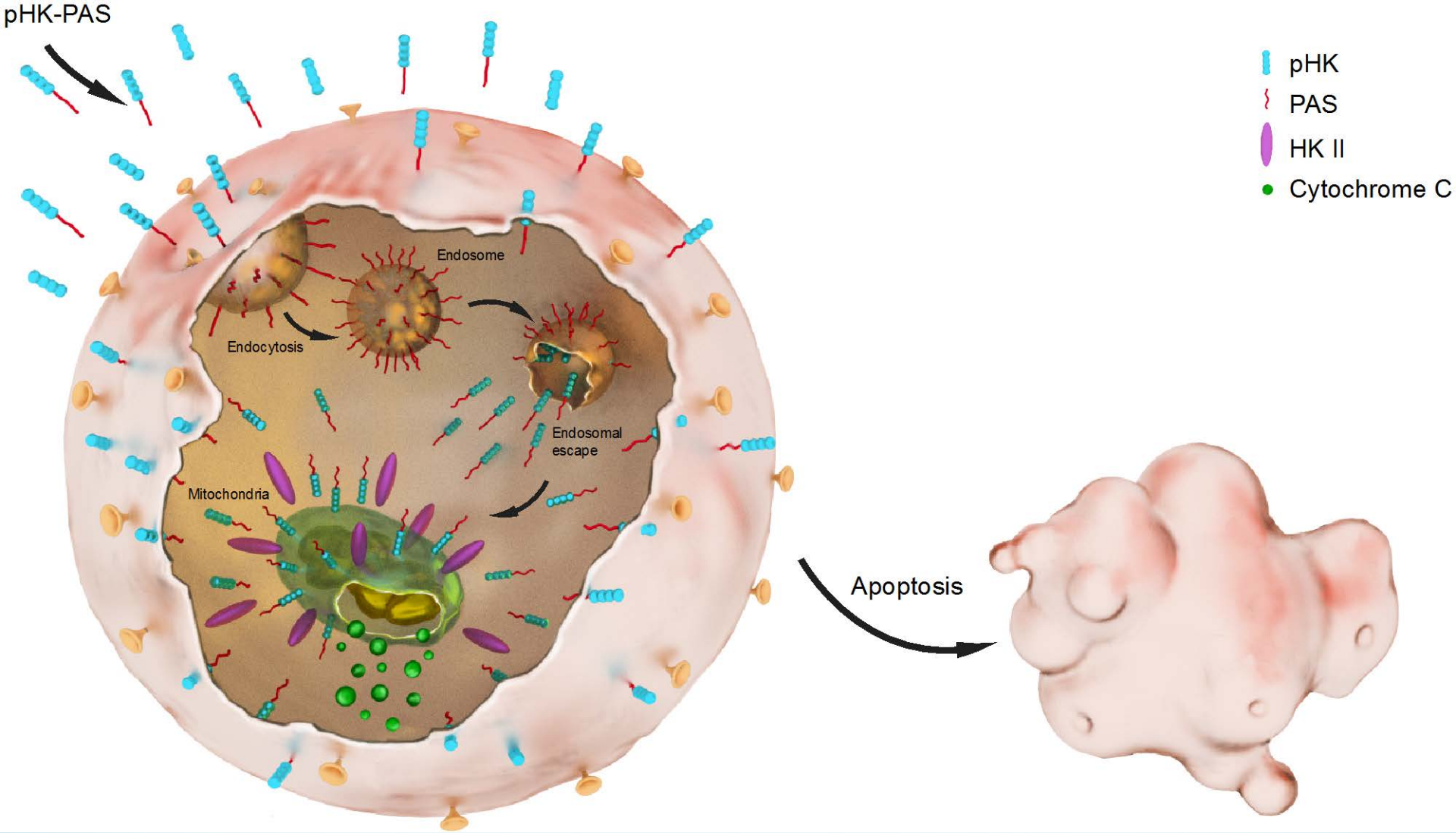
Once in the cytosol, pHK-PAS accumulates at the mitochondrial membrane



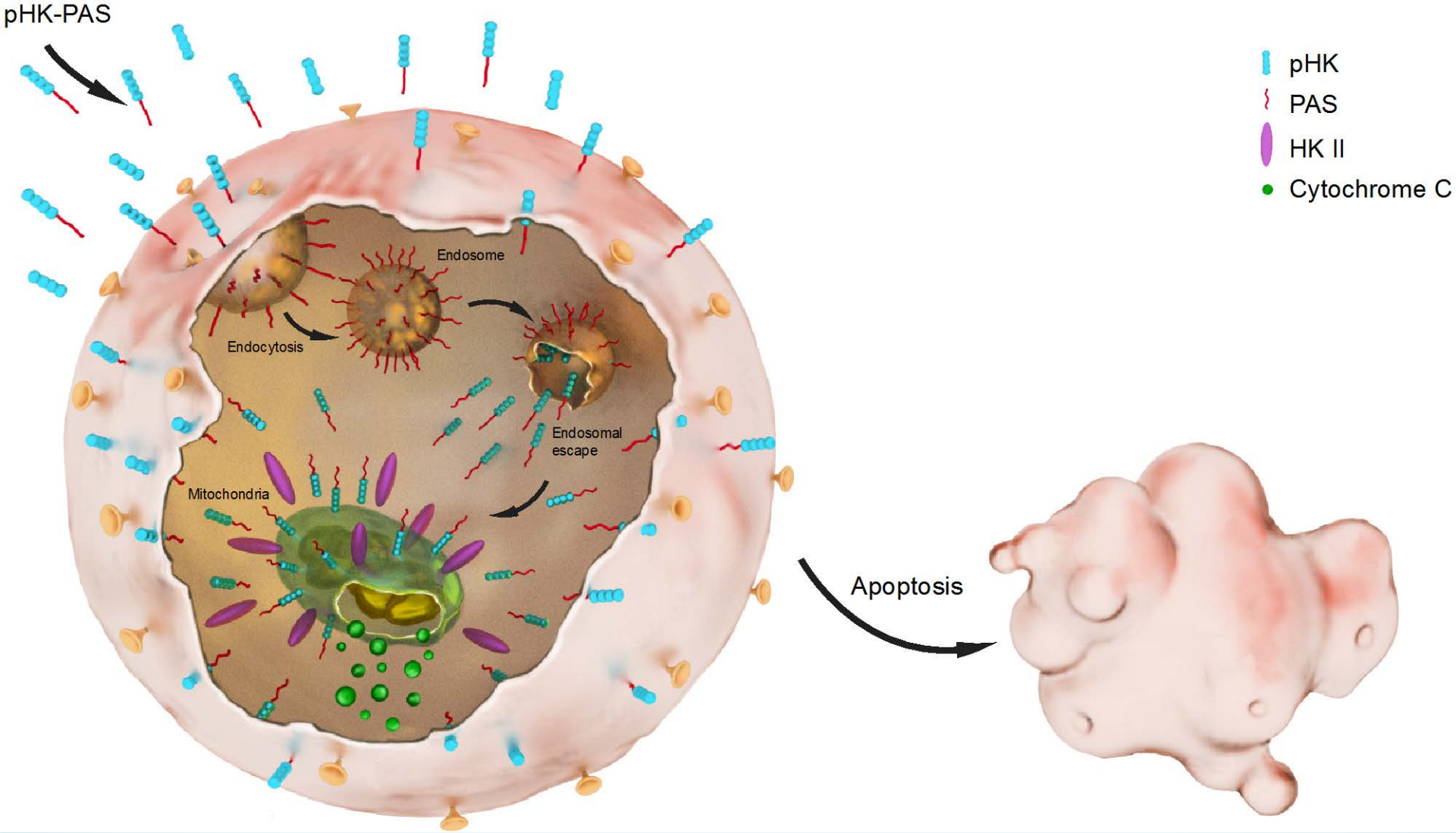
pHK-PAS binds to mitochondria, displacing full-length endogenous HKII in the process.



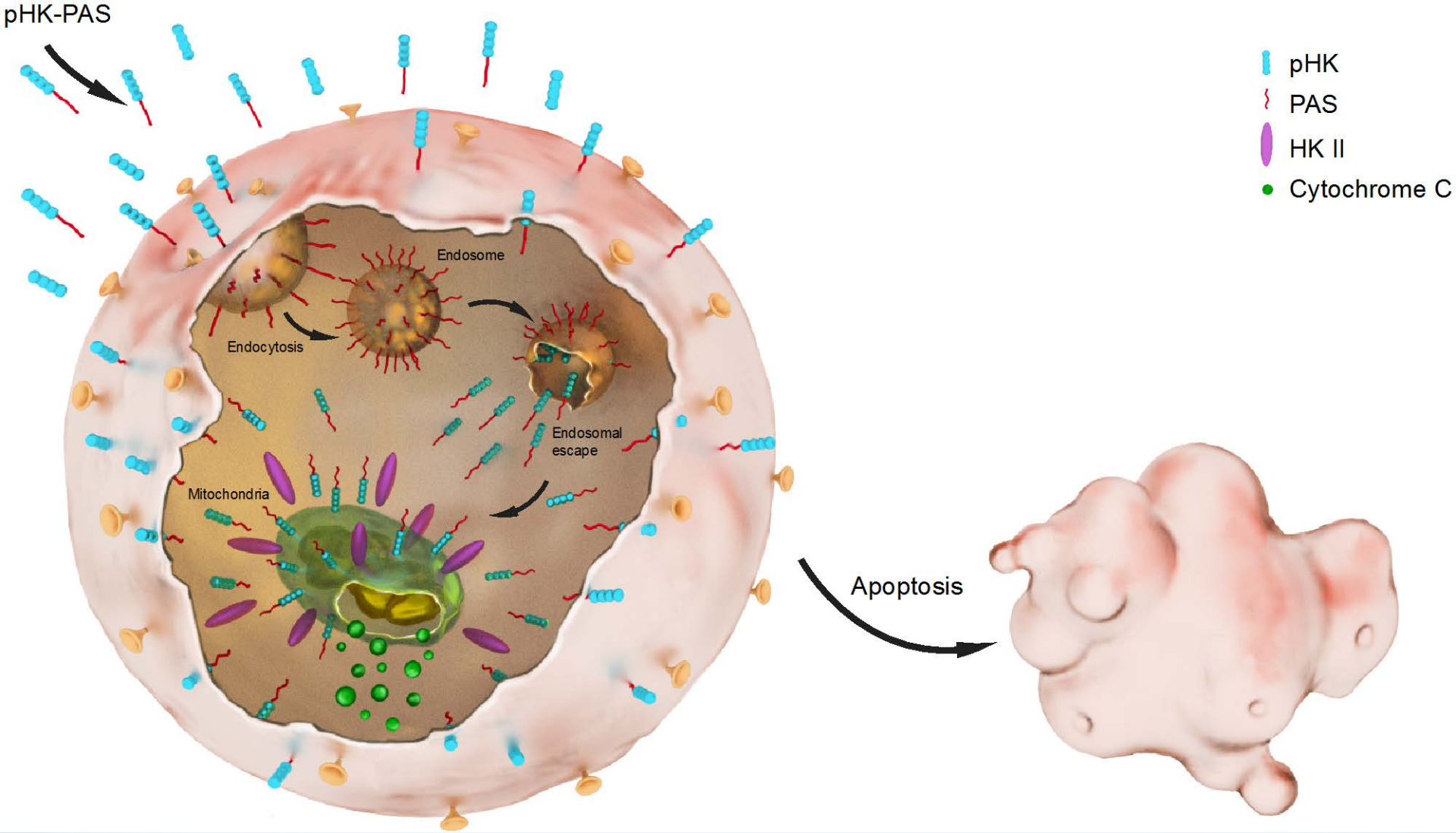




Disruption of the HKII–mitochondria interaction leads to $\Delta\Psi_m$ depolarization, inhibition of mitochondrial respiration and glycolysis and depletion of intracellular ATP levels.

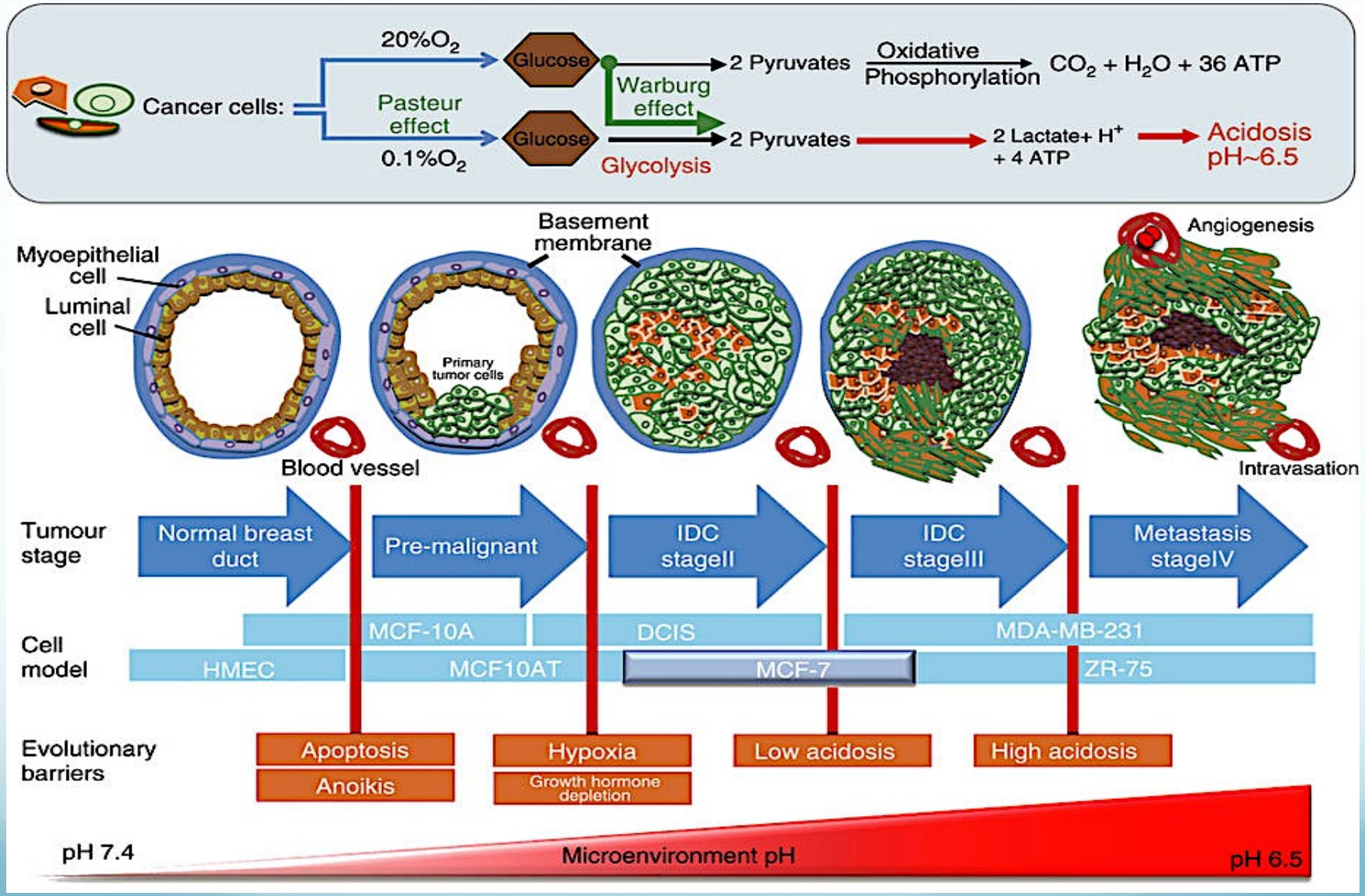


This is followed by release of cytochrome c and, finally, apoptosis.



Significantly, treatment of non-cancer HEK-293 cells with pHK-PAS results in a comparatively negligible loss of cell viability, suggesting that the peptide exhibits selective HKII-mediated cytotoxicity against cancer cells.

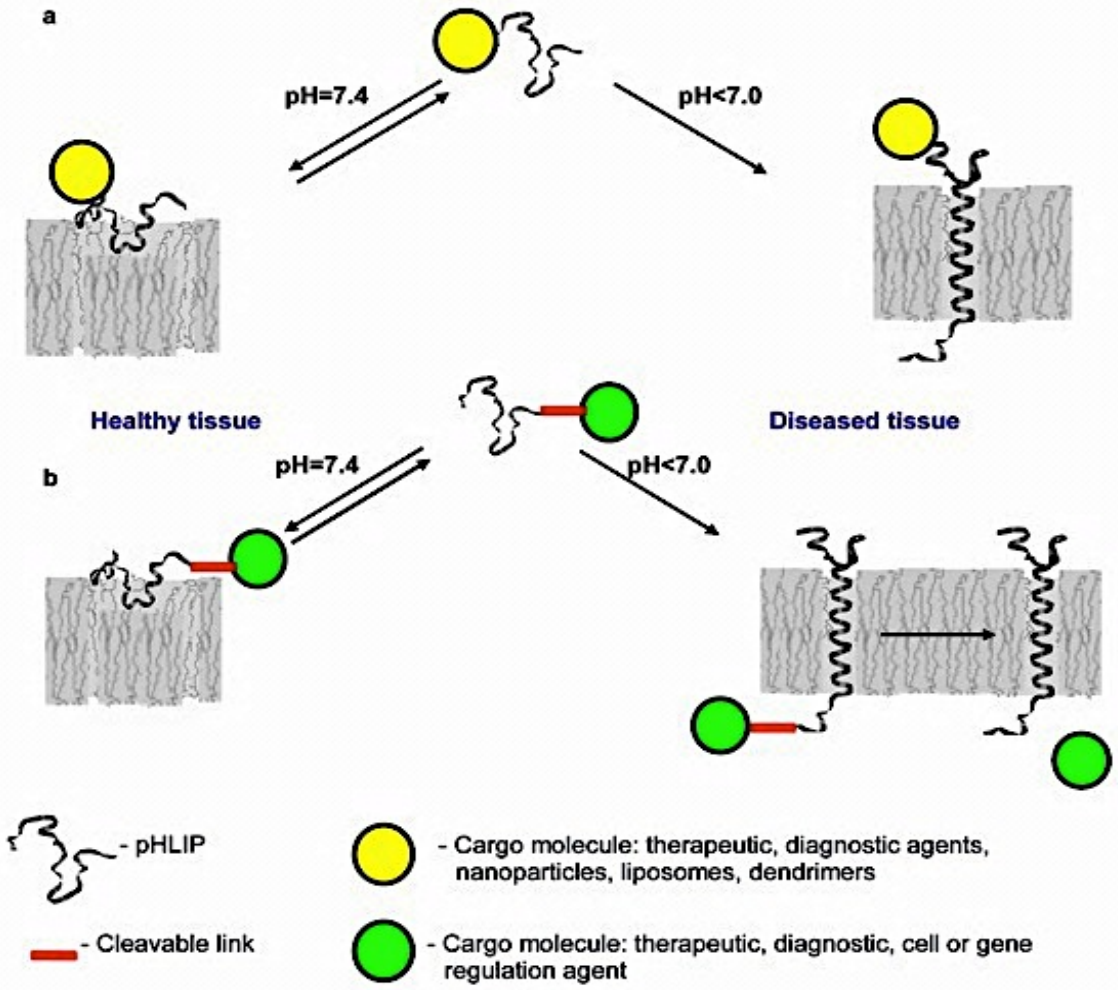
Utilizing the acidic tumor microenvironment

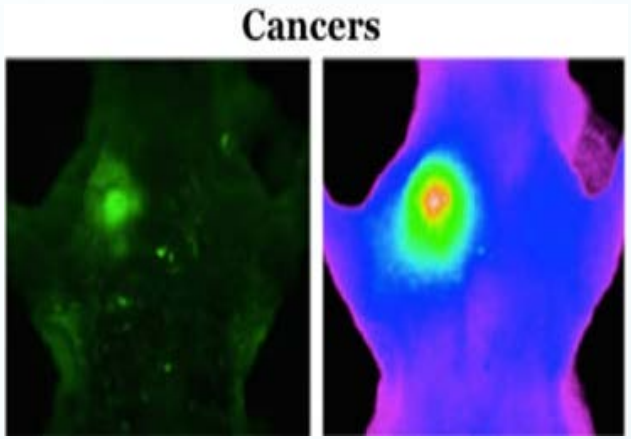


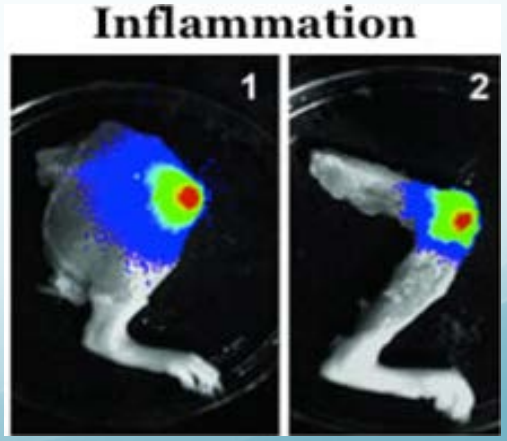
(Damaghi et al., 2015)

pH-sensitive peptides

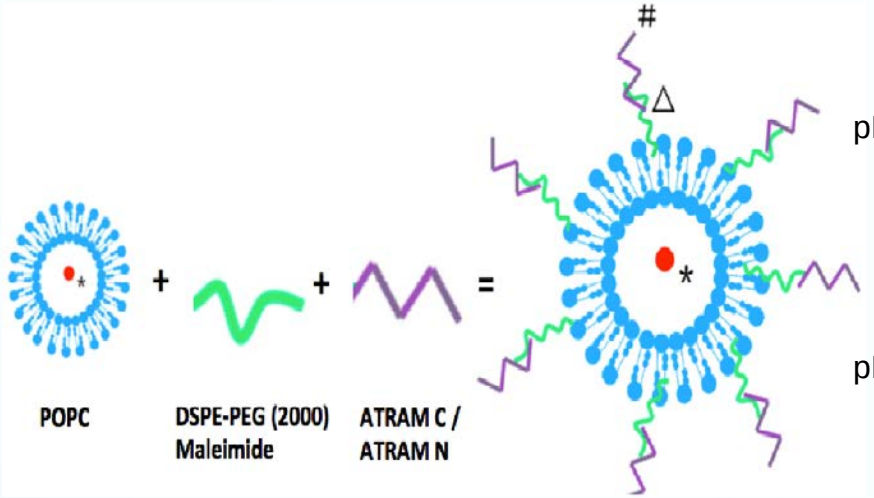
pHLIP (pH-low insertion peptide)
ATRAM (acidity-triggered rational membrane peptide)



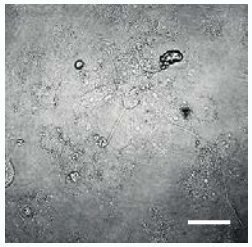




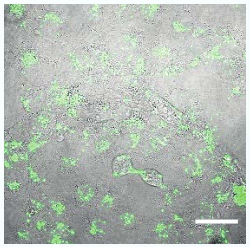
ATRAM-mediated delivery of cancer therapeutics



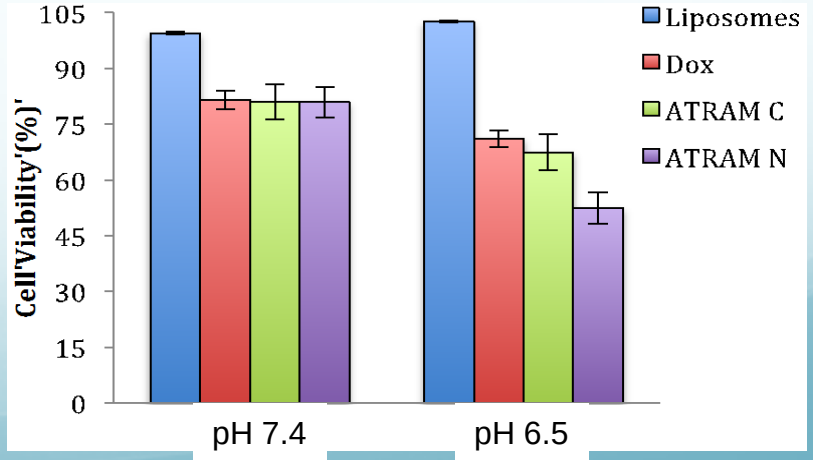
pH 7.4



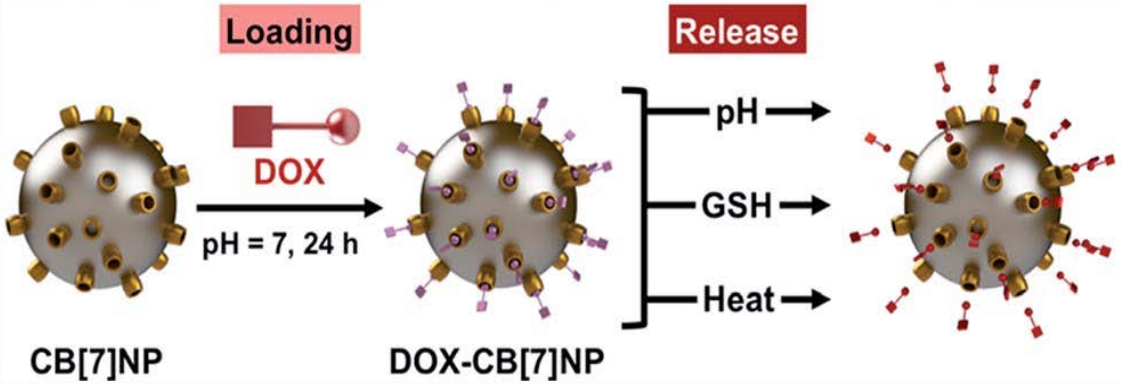
pH 6.5

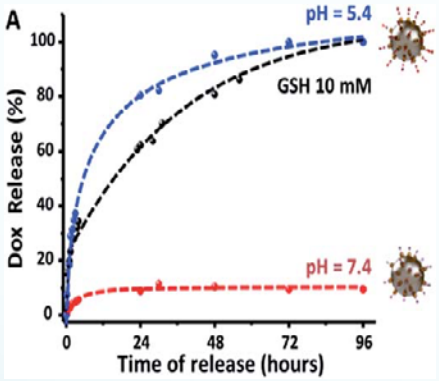


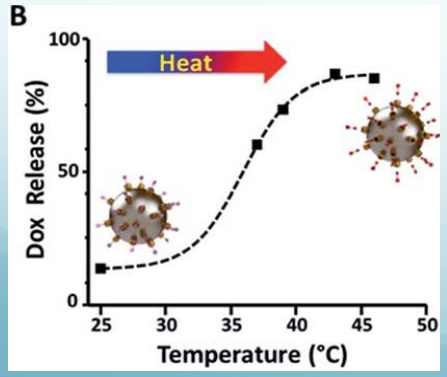
ATRAM-functionalized liposomes exhibit enhanced uptake and cytotoxicity at acidic pH.

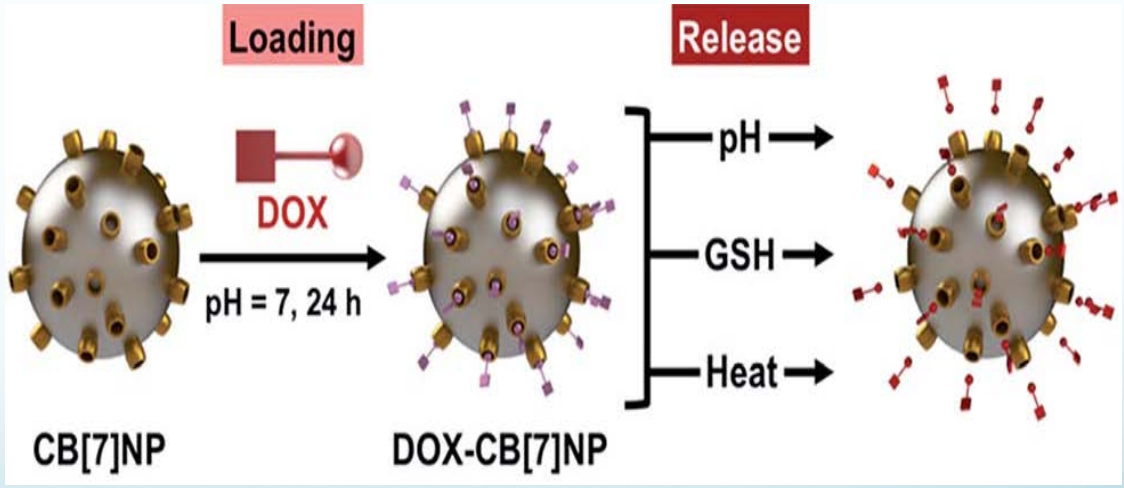


CB[7]-modified iron-oxide nanoparticles



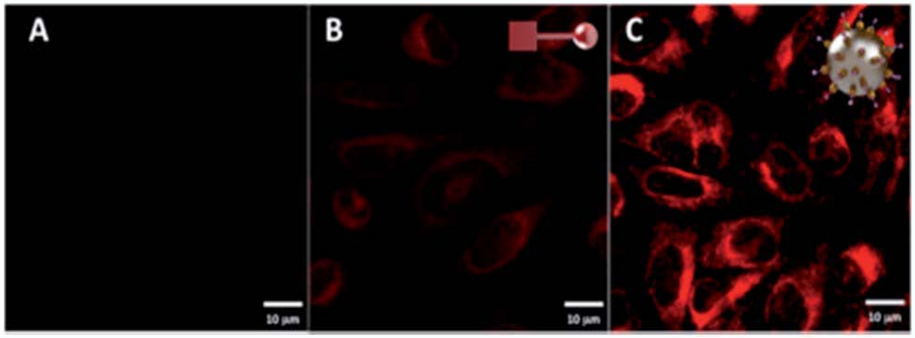




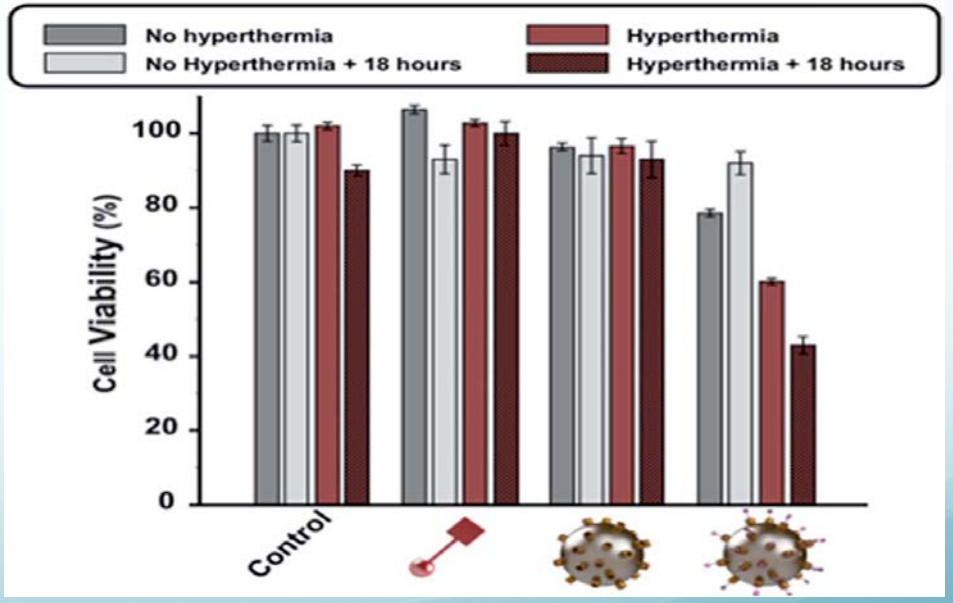


CB[7]-modified iron-oxide nanoparticles

Uptake of Dox vs Dox-loaded nanoparticles in HeLa cells

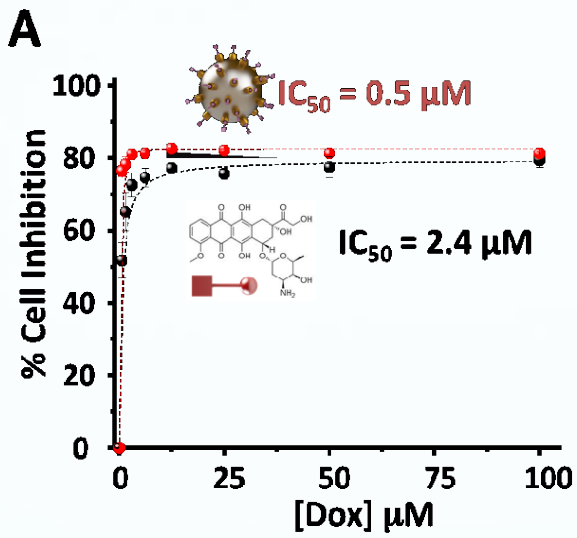


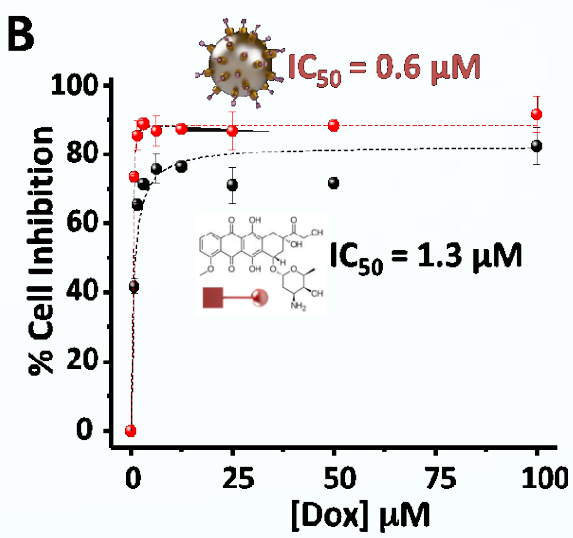
HeLa cell viability in response to hyperthermia and chemotherapy



CB[7]-modified iron-oxide nanoparticles

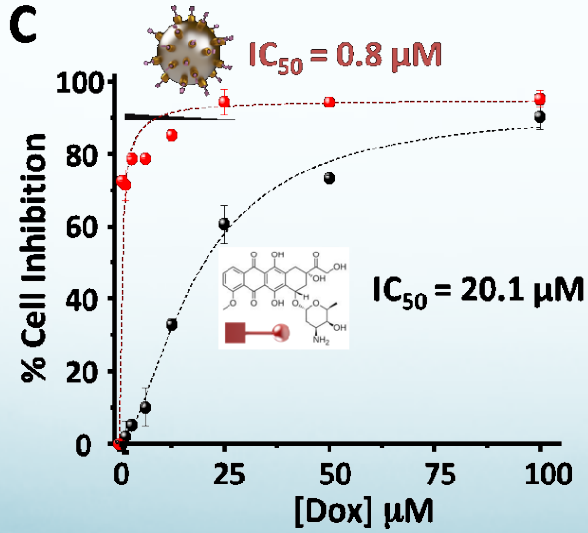
HeLa

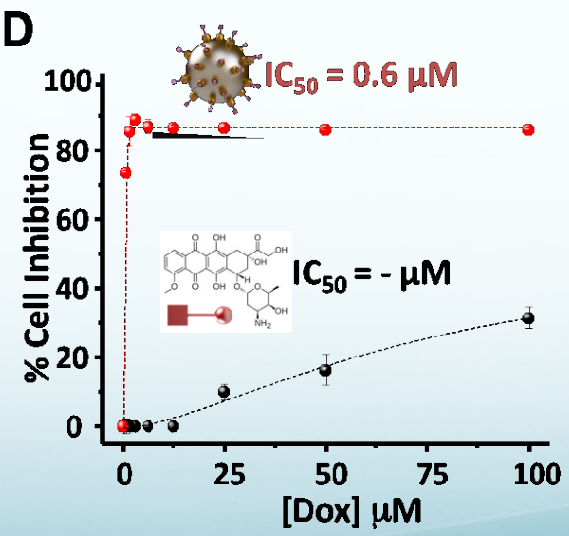




MCF-7

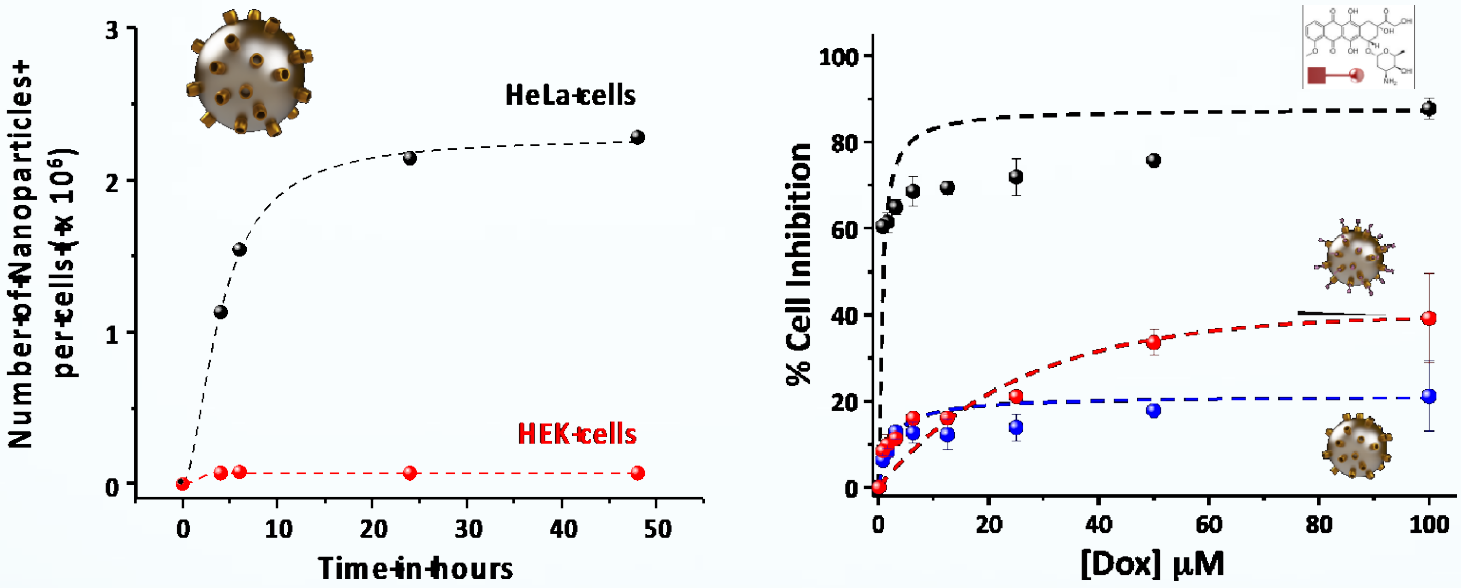
A2780





Dox-resistant A2780

CB[7]-modified iron-oxide nanoparticles



The nanoparticles exhibit substantially lower uptake and cytotoxicity in non-cancerous HEK-293 cells.

Summary

- Development of cancer-specific therapeutics and drug delivery platforms by taking advantage of unique cancer/tumor properties:
  - elevated glycolytic rates (overexpression of hexokinase 2)
  - extracellular/microenvironment acidity

- This serves to:
  - enhance the efficacy of therapeutics/drug delivery platforms
  - reduce targeting of healthy tissue (minimizing side-effects)

Acknowledgements

MagzoubLab
Abiy Woldetsadik
Anja Henning-Knechtel
Sumaya Al Hosani
Ibrahim Chehade
Mona Kalmouni
Sarah Hassan
Maria Vogel
Hadi Saleh

NYU Abu Dhabi
Wael Rabeh
Ali Trabolsi

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